Coexisting fluid phases in model membranes and biological membranes

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Some of the most important, yet unresolved questions in modern cell membrane biology are how cell membranes laterally organize and how they can keep their integrity despite the vigorous membrane trafficking, and thus which mechanisms lead to the sorting of membrane components into those which exit the host membrane and those which are retained.

Submicroscopic lateral membrane domains, often metaphorically termed "rafts", are proposed by cell membrane biologists to function as membrane signaling platforms, and to modulate membrane sorting and trafficking. This raft hypothesis is based on a biochemical assay, the treatment of membranes with cold non-ionic detergent, which casts some doubts on the possibility to use this assay to examine the involvement of lateral membrane domains in fundamental cell membrane processes.

If cell membranes are laterally inhomogeneous, the question arises how membrane domains participate in shaping the membrane, to form buds, tubules, and interconnected networks. Curvature gradients are likely to couple to composition gradients, thus providing a way to laterally segregate membrane components. Furthermore, membrane transport processes often involve the formation of transport vesicles, with membrane compositions different from the overall composition of the donator membrane. In addition to the well established clathrin-dependent endocytic pathway, for example, a non-clathrin dependent pathway has been postulated that involves membrane rafts. The examination of the coupling between curvature and composition, is therefore of prime importance to be able to understand fundamental membrane trafficking processes. Theories have predicted the detailed coupling between membrane heterogeneity and membrane geometry, but it has been difficult so far to validate those theories. Our approach therefore is at the interface between cell biological and purely theoretical approaches, and, as is shown below, has provided key results to validate theoretical predictions. We also describe below how we recently have started to extend the research on model systems, to plasma membrane vesicles, which contain integral and peripheral membrane proteins.

In our opinion, the phenomenon of lateral cell membrane heterogeneity has to be examined from a more rigorous point of view, to be able to formulate hypotheses that are in accordance with physicochemical principles. Model systems with well defined lipid mixtures, provide a first step in this direction.

(a) (b)

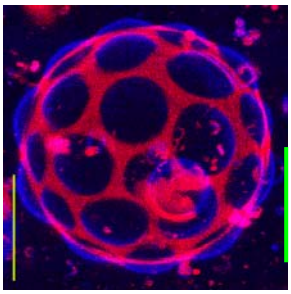

Figure 1. Giant vesicles with Ld \ Lo fluid phase coexistence. Left (a): composition referring to left half of the two-phase region shown in Figure 1. Right (b): Vesicle composition referring to right half of the two phase region shown in Figure 1. Blue: L_o phase, red L_d phase. Scale bars: 5 μm.

Fluorescence microscopy of giant vesicles composed of a lipid mixture that refers to coexistence of a fluid ordered phase (L_o) and a fluid disordered phase (L_d) was performed with a L_d preferring dye; Lissamine rhodamine DOPE, and an L_o phase preferring probe; perylene. Vesicles with low sphingomyelin concentration show disconnected L_o phase spots in a continuous L_d phase matrix (Figure 1a), whereas vesicles with high sphingomyelin concentration (right region of the two-phase ellipse) show disconnected L_d phase spots in a continuous L_o phase matrix (Figure 1b).

Interestingly, domains in vesicles with fluid phase coexistence are observed to have circular shapes. This round domain shape indicates considerable line tension, the one-dimensional analog of surface tension, resulting from an energetic penalty associated with the phase boundary. This line tension has been proposed in the early 1990's, to lead to out of plane curvature and vesicle fission. Figure 2 indicates that we were able to confirm this prediction.

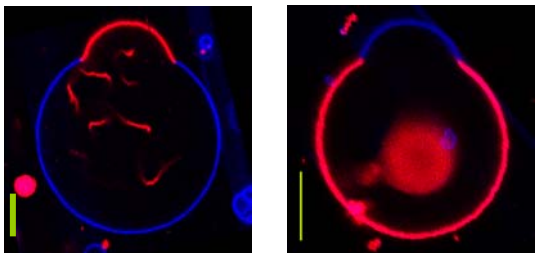

Figure 2. Equatorial section of phase separated vesicles with fluid phase coexistence, obtained by two photon microscopy. Vesicle shapes are considerably deformed from a vesicle with homogenous membrane. Namely, high curvature is found near the phase boundary, indicating line tension to deform these axially symmetric vesicles.

Line tension is observed to deform membranes in a characteristic way. The tendency to reduce the boundary perimeter causes the vesicle to assume a "double bubble" shape, with high curvature in the region near the phase boundary. A close examination of vesicle geometries similar to those shown in Figure 2 allowed us for the first time to a) estimate the magnitude of line tension in phase separated bilayer membranes, b) to determine differences in elastic moduli of liquid ordered and disordered phase, and c) to demonstrate the influence of the resistance towards Gaussian curvature, a phenomenon that is often neglected in the mechanical description of model and biological membranes.

The results described above, allowed confirming predictions from elastic theories dealing with membranes relevant to biological cells. The concepts that are emerging from this research will allow to rigorously test models and hypotheses from cell membrane biology and theoretical membrane biophysics, and will contribute to devising new and more accurate descriptions of fundamental cell biological processes, such as membrane

trafficking and membrane component sorting. To this end, we have recently succeeded in demonstrating microscopically visible phase coexistence in plasma membrane blebs derived from rat basophilic leukemia cells. This experimental approach will be useful in examining protein partitioning in membranes that are more related to membranes under physiological conditions, than the results obtained from the assay of detergent extraction, the widely used defining assay for membrane rafts.